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Cognitive and disease-modifying effects of 11 β -hydroxysteroid dehydrogenase type 1 inhibition in male Tg2576 mice, a model of Alzheimer's disease

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31 SPW have consulted for pharmaceutical companies developing selective 11 β -HSD1 inhibitors.

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Abstract

Chronic exposure to elevated levels of glucocorticoids has been linked to age-related cognitive decline and may play a role in Alzheimer's disease. In the brain, 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) amplifies intracellular glucocorticoid levels. We show that short term treatment of aged, cognitively impaired C57BL/6 mice with the potent and selective 11 β -HSD1 inhibitor UE2316 improves memory, including following intracerebroventricular drug administration to the CNS alone. In the Tg2576 mouse model of Alzheimer's disease, UE2316 treatment of mice aged 14 months for 4 weeks also decreased the number of beta amyloid (A β) plaques in the cerebral cortex, associated with a selective increase in local insulin-degrading enzyme (involved in A β breakdown and known to be glucocorticoid-regulated). Chronic treatment of young Tg2576 mice with UE2316 for up to 13 months prevented cognitive decline, but did not prevent A β plaque formation. We conclude that reducing glucocorticoid regeneration in the brain improves cognition independently of reduced A β plaque pathology, and that 11 β -HSD1 inhibitors have potential as cognitive enhancers in age-associated memory impairment and Alzheimer's dementia.

66 **Introduction**

67 Glucocorticoids have long been recognised to impact on cognitive function, especially with aging (1-
68 3). Older individuals who exhibit learning and memory impairments have elevated glucocorticoid
69 levels that parallel both cognitive deficits and shrinkage of the hippocampus, a key locus for memory
70 formation. The hippocampus expresses a high density of corticosteroid receptors, both the lower
71 affinity glucocorticoid receptor (GR) and the higher affinity mineralocorticoid receptor (MR), and
72 these receptors are also abundant in other neocortical regions associated with cognition (4). Elevated
73 glucocorticoid concentrations in vitro and in vivo promote biochemical, electrophysiological and
74 structural changes in hippocampal neurons, which associate with poorer memory formation (5, 6).
75 Manipulations which maintain low glucocorticoid levels from birth (neonatal programming) or mid-
76 life (adrenalectomy and low dose steroid replacement) prevent the emergence of cognitive deficits
77 with age (7).

78 Some patients with dementia, including those with Alzheimer's disease (AD), have elevated
79 circulating cortisol levels, which may contribute to AD pathogenesis (8, 9). It has been postulated that
80 excess glucocorticoids increase levels of amyloid precursor protein (APP) and APP cleaving enzyme
81 (BACE) leading to increased amyloid A β formation, reduced A β degradation via attenuation of
82 insulin degrading enzyme (IDE) and increased tau expression (10). Other relevant glucocorticoid
83 actions include hyperglycemia/insulin resistance, angiopathic and anti-angiogenic actions, increased
84 excitatory (NMDA) neurotransmission and post-synaptic calcium signaling promoting neurotoxicity,
85 metabolic endangerment of neurons and deleterious alterations in neuroimmune function (11).

86 Glucocorticoid action via intracellular MR and GR is determined not only by circulating steroid levels
87 but also by target tissue concentrations, modulated by intracellular metabolism by the isozymes of
88 11 β -hydroxysteroid dehydrogenase (11 β -HSD) (12). The adult forebrain expresses 11 β -HSD type 1,
89 which catalyses conversion of inert 11-keto corticosteroids (cortisone, 11-dehydrocorticosterone) to
90 active cortisol and corticosterone. 11 β -HSD1 levels are increased in the aging rodent hippocampus
91 and cortex and correlate with cognitive decline (13). Transgenic mice modestly overexpressing 11 β -

HSD1 in the forebrain show premature memory decline with aging, while 11 β -HSD1 null mice on two distinct genetic backgrounds, and even heterozygous null mice (with 50% less enzyme) resist cognitive decline with aging in a variety of tests (14). This protection associates with loss of the age-associated rise in intrahippocampal corticosterone levels but without changing plasma corticosterone levels (13).

Treatment of already aged mice with selective 11 β -HSD1 inhibitors improves spatial memory performance. Effects are rapid, occurring within hours to days (15-17). Moreover, in small randomized placebo-controlled trials, the non-selective 11 β -HSD inhibitor carbenoxolone improved memory in healthy aging men and in patients with type 2 diabetes (18). Whilst 11 β -HSD1 inhibition improves glucose homeostasis and other metabolic parameters in obesity, metabolic changes were not correlated with cognitive effects in aged rodents or humans. These results support examination of selective 11 β -HSD1 inhibitors in the treatment of age-related cognitive impairments.

Here we examined a crucial issue, whether selective 11 β -HSD1 inhibition alters cognition and pathology in AD. We used a murine AD model, the well-characterized Tg2576 mouse which bears a mutated human APP gene. We generated and used UE2316, a novel and selective inhibitor of both human and rodent 11 β -HSD1 with a low nanomolar IC₅₀ value and high penetration into the brain (19,20).

Materials and Methods

UE2316

UE2316 ([4-(2-chlorophenyl-4-fluoro-1-piperidinyl)][5-(1H-pyrazol-4-yl)-3-thienyl]-methanone) was synthesized by High Force Ltd, UK according to methods previously described (21). In vitro screening of UE2316 potency in HEK293 cells stably transfected with hsd11b1 (22) showed a greater median inhibitory concentration (IC₅₀) than our previously reported compound UE1961 (15, 20). Inhibition of 11 β -HSD1 activity in tissue extracts was quantified as previously described (22). Liver

brain and white adipose tissues were collected and snap frozen on dry ice. Frozen tissue (50-80mg) was homogenized in 700µl of chilled Krebs Buffer and a cleared homogenate prepared by centrifugation at 3500rpm for 5 minutes. The protein concentration of this homogenate was determined by Bradford assay. For the assay, 25µl of 10mM NADPH was added to 250µg of the homogenate in a final volume of 200µl chilled Krebs buffer and incubated at 37°C for 20 minutes. 3H-cortisone (25µl of 200nM) was then added and the assay incubated for a further 15 minutes prior to termination by rapid freezing on dry ice. ³H-cortisone to ³H-cortisol conversion was determined in 50µl of the defrosted reaction by capturing liberated ³H-cortisol on anti-cortisol (HyTest Ltd)-coated scintillation proximity assay beads (protein A-coated YSi, GE Healthcare). The percentage inhibition was determined by measuring the conversion of ³H-cortisone to ³H-cortisol relative to that in tissue from vehicle treated mice.

Animals

All in vivo experiments were performed under a project license issued under the UK Scientific Procedures (Animals) Act, 1986, and with local ethical committee approval. Male C57Bl/6 mice were obtained from Harlan (UK). Male mice were chosen to eliminate the potential effects of gonadal hormonal fluctuations observed in females. Animals were group-housed under controlled lighting (on 07.00-19.00h) and temperature (22°C), with access to food and water ad libitum. Experimental procedures are summarised in Table 1.

For measurement of pharmacodynamic inhibition following oral administration, oral gavages with vehicle (38% PEG, 2% DMSO, 60% saline; Sigma, Poole, UK) or UE2316 dissolved in vehicle were performed in the morning in animals aged 8-10 weeks (n=3 per dose). Animals were sacrificed post dosing (1, 4 and 6 hours) and the tissues retained for analysis of 11β-HSD1 inhibition.

For assessment of pharmacodynamic inhibition following subcutaneous administration, C57Bl/6 mice (8-10 months, Harlan UK) (n=3 per group) were treated with either vehicle (50:50 DMSO: PEG, Sigma) or 10mg/kg/day UE2316 in vehicle via subcutaneously implanted Alzet osmotic minipumps

(model 2004, Charles River, Margate, UK) for 14 days. Animals were sacrificed at this stage and the tissues retained for analysis of 11 β -HSD1 inhibition.

To show that the effects of UE2316 were not merely due to any peripheral metabolic actions of 11 β -HSD1 inhibition, the agent was administered intracerebroventricularly to aged male C57Bl/6 mice (24 months, obtained from an in-house stock) were treated with either vehicle (artificial CSF; Alzet; Charles River) (n=9) or 100ng/h UE2316 in artificial CSF (n=8) administered via intracerebroventricular (icv) infusion, for 9 days, as previously described (23).

For assessment of the effects of UE2316 on cognition in aging animals, aged male C57Bl/6 mice (22 months, Harlan UK) were treated with either vehicle (n=6), 5mg/kg/day UE2316 (n=8) or 15mg/kg/day UE2316 (n=8) in vehicle (50:50 DMSO: PEG, Sigma) by subcutaneously implanted Alzet osmotic minipumps (model 2004, Charles River, Margate, UK) for 23 days, with body weights monitored at the start and end of the treatment.

For the assessment of the effects of UE2316 in a model of AD, male Tg2576 (Hsiao et al., 1996) and age-matched genetic control (BL6;SJL) littermates were obtained from Taconic Europe (Ry, Denmark). Animals were singly housed due to aggressive behavior. In the short term treatment study, 14 month old mice of each genotype (n=10 per group) were allocated at random to receive either UE2316 (10mg/kg/day) or vehicle (50:50 DMSO: PEG, Sigma) via 2 Alzet osmotic minipumps (model 2004) implanted subcutaneously to provide sufficient volume of drug or vehicle for 29 days. Food intake and body weight were monitored weekly throughout. For the long term study in which UE2316 was administered by incorporation in the diet, 6-7 month old Tg2576 male mice that were screened for eye color, coat color and rd1 homozygosity for the Pde6b^{rd1} retinal degeneration mutation by Taconic were fed with control diet (RM1) (n=16) or with RM1 containing 175 ppm UE2316 (for a calculated dosage of 30 mg/kg/day) (n= 32) (Special Diet Services, Broxburn, UK) for up to 57 weeks. Food intake and body weights were monitored weekly throughout the experiment and drug dosages were calculated based on average daily food intake. Each cohort of mice underwent repeated longitudinal cognitive testing.

168 **Behavior**

169 Mice were acclimatized to the behavior room for at least one hour before all procedures in order to
170 minimize stress. All behavioral testing was conducted during the day between 9am and 12pm.

171 **Memory in passive avoidance**

172 Passive avoidance was assessed over 2 days (for aging studies in C57Bl/6 mice on days 13 and 14
173 after the start of treatment, for the short term UE2316 study in Tg2576 mice on days 27 and 28 and at
174 weeks 15 and 41 in the long term UE2316 Tg2576 study) in a step through light/dark box passive
175 avoidance apparatus (Ugo Basile Comerio, VA, Italy) (13). On the first day, the latency to enter the
176 dark compartment from the light compartment was measured, with the door to the dark compartment
177 opening 30 seconds after the start of the trial. Twenty four hours later, the latency to enter the dark
178 compartment was repeated, which was followed by a mild 0.3mA, 2 second foot-shock in the dark
179 compartment. The mice were then retested 6 hours later for the latency to enter the dark compartment,
180 this time without a foot-shock. The latencies were measured automatically by the device following the
181 opening of the door separating the light and dark compartments with a maximal time allowed of 300
182 seconds. Mice that did not enter the dark compartment were eliminated from analysis.

183 **Y maze**

184 Y maze testing of spatial hippocampal memory was performed as previously described with a 2 hour
185 inter-trial interval (ITI) on day 10 following the start of treatment in the aged UE2316 study (15). For
186 the UE2316 icv administration study, Y maze testing was performed on day 8 of treatment. The
187 amount of time spent in each arm was measured and analyzed using AnyMaze software (Stoelting,
188 Dublin, Ireland).

189 **Open field**

190 The open field test was performed on day 23 of the short term Tg2576 study and week 38 of the long
191 term Tg2576 study. Mice were placed in an open field (OF) box (60 x60 cm) marked off into 16 equal
192 squares. The outer row of squares adjacent to the walls of the box are considered less anxiogenic than

the inner squares. For a 5 min period, the number of crossings, time, and distance (movement of all four legs into a new square) into each square was noted. Total movement in the maze reflects general activity and the relative movement in the inner zone is correlated to the anxiety state of the mouse.

Spontaneous alternation

Spontaneous alternation, a test of working hippocampal memory, was tested after 26 days of treatment in the short term treated Tg2576 mice and after 39 weeks of treatment in the long term diet study. Mice were placed in a Y maze apparatus consisting of three enclosed black Plexiglas arms (50 cm long, 11 cm wide and 10 cm high), with prominent extramaze visual cues. Mice were allowed to explore the maze for five minutes after starting in a randomly chosen 'start' arm. The order and number of arm entries by each mouse in the 5 minute test period was recorded. Percentage spontaneous alternation was calculated using the following formula: % spontaneous alternation= [number of alternations (which is entries into 3 different arms consecutively)/(number of arms entered minus 2)] x 100.

Morris water maze

Morris water maze testing was performed as previously described on day 14 of the short term study and week 52 of the long term study (24). For the short term treatment study in Tg2576 mice, mice who did not swim (i.e. did not engage with the task) during the visible platform test, in which a visual cue (i.e. stacked Lego blocks) was placed on the submerged platform in the tank and no visuospatial clues were present (curtains were closed) were eliminated from analysis. The mice undertook 4 trials per day with a 20 minute inter trial interval and a maximum swim time of 90 seconds per trial for 4 days. Latency, swim speed and the percentage of time spent in each quadrant of the pool were measured by Watermaze software (Actimetrics, Evanston, IL, USA). In the long term treatment study, mice were initially assessed after 12 months of treatment for their ability to engage in the visible platform test. In this instance, mice that were able to find the platform and improve their latencies after 2 days of 4 x 90 second trials were then tested in the spatial water maze, in which the platform remained submerged without a visual cue on top and the mice utilised spatial clues located around the

maze (curtains open). Mice were tested in 4 x 90 second trials per day over 6 days. Twenty four hours after the final spatial water maze trial, the mice were then tested in the 90 second probe test, in which the hidden platform was removed from the tank and the percentage of time spent swimming in the target quadrant was measured.

After behavioural testing, mice were sacrificed by cervical dislocation on day 29 of vehicle or UE2316 treatment in the short term study or after 44 and 57 weeks in the long term study. The brains were removed and hemisected coronally. One half of the brain was dissected and cortex, hippocampus and cerebellum were immediately frozen on dry ice and stored at -80°C for further analysis. The other half was fixed in 4% paraformaldehyde in PBS (4% PFA) (VWR, Lutterworth, UK) and cryoprotected in 30% sucrose (Sigma) overnight at 4°C before storage at -80°C for immunohistochemistry.

Immunohistochemistry

All immunohistochemistry was performed on free floating 25 µm sections stored at -20°C in cryoprotectant (50 mM phosphate buffer, 25% glycerol, 25% ethylene glycol, Sigma). Sections were transferred to a 12 well tissue culture plate with Netwell inserts (VWR) and washed in PBS. Antigen retrieval was performed by heating the sections in sodium citrate buffer, pH 6.0 (Sigma) at 95°C for 15 minutes, followed by peroxidase treatment (1% H₂O₂ in PBS; Sigma) for 30 minutes to remove endogenous peroxidase activity, washed and then blocked with the appropriate serum for 1 hour followed by overnight incubation at 4°C with the antibody of choice. For staining using the 6E10 antibody for visualization of amyloid beta plaques, the sections were blocked using the mouse on mouse (MOM) Ig blocking reagent (Vector Laboratories, Peterborough, UK) followed by overnight incubation with a 1:1000 dilution of beta amyloid 1-16 mouse monoclonal antibody (6E10) (Covance, Cambridge Bioscience, Cambridge, UK). Following washing, sections were incubated with secondary antibody for 1 hour at room temperature. Staining was visualised using the Vectastain ABC kit and DAB peroxidase substrate kit (Vector Laboratories). The sections were then mounted on Superfrost Plus slides (VWR), dehydrated and coverslipped. The number of 6E10 positive plaques per brain area

was counted by an experimenter blinded to the treatment group using a Zeiss Axioskop and the KS300 imaging program (Zeiss, Eching, Germany). Plaque area, measured using the same program, was expressed as plaque area divided by the total area of the brain region. Iba-1 antibody was purchased from Abcam (Cambridge, UK). Goat and rabbit serum were purchased from Sigma. Biotinylated rabbit anti-goat IgG antibody and biotinylated rabbit anti-sheep IgG antibody were purchased from Vector Labs.

Western blotting

Protein extracts were prepared from brain areas by homogenization in Krebs buffer containing protease inhibitor (Roche, Burgess Hill, UK) followed by centrifugation at 3000 RPM for 5 minutes. Protein concentration of the supernatant was measured using the Bradford Assay (BioRad, Hemel Hempstead, UK). Proteins were separated by SDS-PAGE using NuPAGE Novex 4-12% bis-tris gels (Invitrogen, Paisley, UK) and transferred to nitrocellulose membranes (0.2µm pore size; Invitrogen). Membranes were blocked for 1 hour at room temperature in 5% non-fat dry milk blotting grade blocker (BioRad) in PBS, pH 7.4, containing 0.1% Tween 20 (PBS-T) and then incubated overnight with shaking at 4°C with the primary antibody diluted in blocking reagent. This was followed by incubation at room temperature in the appropriate secondary antibody. IDE, PSD95, ADAM10, synaptophysin and CD31 antibodies were purchased from Abcam. The anti-BACE 1 N terminus (46-62) antibody was sourced from Sigma. Mouse beta-tubulin antibody was purchased from Merck-Millipore (Watford UK). Goat-anti rabbit IgG antibody was obtained from Licor Biosciences UK (Cambridge UK). Alexa Fluor 680 donkey anti-sheep IgG (H+L) and Alexa Fluor 680 rabbit anti-goat IgG antibodies were purchased from Invitrogen (Paisley, UK). Proteins were visualized and band intensities were quantified using the Odyssey Infrared Imaging System (LiCor Biosciences UK, Cambridge, UK).

Statistical analysis

Data are expressed as mean \pm SEM. Groups were compared by ANOVA. When ANOVA was significant post hoc tests were performed as indicated in the Figure legends. Differences were considered significant when $p < 0.05$.

Results

UE2316 inhibits 11 β -HSD1 in the brain

We previously reported the discovery and pharmacological effects of the selective 11 β -HSD1 inhibitor UE1961, which was based on a thiophene amide scaffold (15). However, this molecule has sub-optimal potency and pharmacokinetic properties for progression to late-stage preclinical development. Further medicinal chemistry optimisation of this compound, replacing the decahydroquinoline and substituted piperidine groups flanking the thiophene core, led to the identification of UE2316 (Figure 1A). UE2316 displays greater potency than UE1961, excellent selectivity and an improved drug metabolism and pharmacokinetic profile for use in in vivo studies (Figure 1B). Pharmacodynamic inhibition of 11 β -HSD1 in tissues was confirmed following administration by oral and subcutaneous (SC) routes. Single dose oral administration of UE2316 to C57BL/6 mice induced significant ex vivo inhibition of 11 β -HSD1 in the brain for at least 4 hours (Figure 1C), while constant infusion of 10mg/kg/day of UE2316 over 14 days by SC Alzet osmotic minipumps also produced 34.2 ± 8.3 % inhibition of 11 β -HSD1 in the brain (data not shown). The results from these studies were in agreement with those from previous studies (19,20). UE2316 was thus chosen to investigate the effects of chronic 11 β -HSD1 inhibition on cognitive impairment and AD pathology in mouse models using either SC or oral administration.

UE2316 acts in the brain to improve cognition in aged wild type mice

To assess the effects of UE2316 on memory in cognitively impaired mice, C57BL/6 male mice aged 22 months were randomly assigned to treatment with 0, 5 or 15 mg/kg/day of UE2316 via SC implanted Alzet minipumps for 14 days. In the Y-maze, a non-stressful test of hippocampal-associated spatial memory, there was a significant increase in time spent exploring the novel arm after a 120 min inter-trial interval in mice receiving 15 mg/kg/day UE2316 compared to vehicle-treated

controls (Figure 2A). Cognition was also assessed in the passive avoidance task, which tests emotional and fear associated memories (13). During the retention phase of the passive avoidance test, UE2316 increased latency to enter the dark compartment at both 5 and 15 mg/kg/day compared with vehicle-treated mice, indicating improved memory (of the footshock) (Figure 2B).

To investigate whether brain-specific inhibition of 11 β -HSD1 was responsible for these improvements in cognition, we delivered UE2316 directly to the brain of aged (24 month old) C57BL/6 mice at an appropriate concentration via icv administration for 9 days. Post-mortem analysis of whole brain samples revealed 39.9 ± 5.5 % inhibition of 11 β -HSD1 was achieved with a 100 ng/h infusion. Vehicle-treated aged controls showed impaired spatial memory in the Y maze (similar times spent in all 3 arms) as previously reported (15). Mice treated with icv UE2316 spent more time exploring the novel arm of the Y-maze than vehicle-treated mice, indicating an improvement in spatial memory after 8 days of treatment (Figure 2C).

UE2316 improves cognition in a murine model of Alzheimer's disease

Following confirmation of the effects of 11 β -HSD1 inhibition using UE2316 in age-related cognitive impairment, we examined effects of short-term UE2316 administration in the Tg2576 mouse model of AD. Tg2576 mice carry a transgene with mutations at amino acids 670 and 671 in the human APP gene under the control of the hamster prion promoter, which leads to accumulation of A β plaques in the brain from 9-12 months of age with consequent cognitive impairment (25). Singly housed age-matched male mice were separated into 4 groups of 10 mice per group: wild type or Tg2576 mice administered vehicle or UE2316. Mice were treated by 2 SC implanted Alzet minipumps from 14 months of age for 29 days. No treatment-related adverse effects on final body weight, daily food intake or adrenal size were observed. However, as previously reported, Tg2576 mice weighed less than their wild type littermates throughout the study despite consuming more food (Supplementary Figure 1A-B), which may reflect their increase in locomotor activity and hypothalamic dysfunction (26,27).

Tg2576 mice perform poorly in the Y-maze spatial memory test due to retinal degeneration, therefore, fear-associated memory was assessed in the passive avoidance task, which is not dependent on visual acuity. In this test, performed on days 27 and 28 of drug or vehicle administration, UE2316 treatment increased latencies in re-entry to the dark compartment at 6 hours post footshock in both control and Tg2576 mice (Figure 3A), suggesting an improvement in fear associated memory with drug treatment. The effect of UE2316 treatment was particularly pronounced in Tg2576 mice, which may reflect a difference in sensitivity to the electrical shock in this mouse strain. In a separate open field test, no difference was observed in the time spent in the inner zone in either wild type or Tg2576 mice with or without drug (two way ANOVA, treatment effect: $p=0.98$), suggesting that UE2316 does not affect anxiety (Figure 3B). No difference in speed was observed between either strain, in the presence or absence of drug (two way ANOVA, treatment effect: $p=0.94$, data not shown).

In spontaneous alternation, a test of working hippocampal memory, Tg2576 mice tended to enter more arms than the control mice ($p=0.06$), which again may be due to their increased locomotion (data not shown) (26). Treatment with UE2316 led to a trend in increased percentage alternation in both wild type and Tg2576 mice compared to vehicle (two way ANOVA, treatment effect: $p=0.06$) (Figure 3C).

UE2316 prevents cognitive decline in a murine model of Alzheimer's disease

Effects of long-term UE2316 treatment were examined by administering UE2316 in the diet to 6-7 month old Tg2576 mice over a period of 57 weeks. The mice on the UE2316-supplemented diet maintained an average daily dose of approximately 30 mg/kg/day throughout the experiment and did not exhibit any adverse effects (Supplementary Figure 2A). UE2316-treated mice tended to weigh less than vehicle-treated mice up to 44 weeks (ANOVA, $p<0.01$) despite eating more food (ANOVA, $p<0.001$) (Supplementary Figure 2B-C). In contrast to the short-term study, mice were pre-screened for retinal degeneration (RD) and only those which were RD negative were included. Memory was assessed at intervals using passive avoidance, spontaneous alternation and Morris Water Maze tests.

Fear associated memory was assessed using the passive avoidance test after 15 and 41 weeks of treatment. As expected, at 15 weeks, when mice were aged 10-11 months, similarly increased latencies were observed following training in both the vehicle and UE2316-treated groups, consistent with preserved cognitive function at this age (Figure 4A). In contrast, after 41 weeks of treatment, when the mice were aged 16-17 months, vehicle-treated mice exhibited cognitive impairment as demonstrated by lack of prolongation of latency after training, but Tg2576 mice treated with UE2316 maintained an increase in latency to enter the dark compartment 6 hours post shock, indicating that UE2316 prevents an age-associated decline in fear associated memory (Figure 4B). There was an increase in latency for training in vehicle-treated mice, suggesting an impairment in their ability to effectively to engage with the task. No difference in anxiety was observed with UE2316 treatment in a separate open field test (two way ANOVA, treatment effect: $p=0.25$), but Tg2576 mice were less anxious compared to wild type mice (two way ANOVA, genotype effect: $p<0.01$) (Figure 5A). There was also an increase in locomotion in Tg2576 mice when compared to wild type mice (two way ANOVA, genotype effect: $p<0.01$), but no effect of treatment (two way ANOVA, treatment effect: $p=0.69$).

Working memory was assessed at 39 weeks of treatment using the spontaneous alternation test. UE2316 increased spontaneous alternation (Figure 5B), although there was no difference in the total number of arm entries (data not shown).

Spatial memory was assessed in the Morris Water Maze after 52 weeks of treatment (mice aged 18-19 months). Swim speeds were not affected by UE2316 treatment and there was no difference between strains (data not shown). UE2316 reduced the time taken to find the hidden platform across the testing period (Figure 5C), and increased time spent in the target quadrant during the probe test performed 24 hours after the final trial (vehicle: $34.2\pm3.2\%$, UE2316: $52.1\pm5.1\%$, $p=0.02$), consistent with improved spatial learning and retention (Supplementary Figure 3A).

Effect of UE2316 on A β plaques in the brain

370 Immunohistochemistry with 6E10 antibody (28), which detects A β 1-16, was performed on Tg2576
371 brains to determine whether UE2316 affected the number and volume of A β plaques (wild type
372 control brains were not analysed as these mice do not develop amyloid plaques) (Figure 7A).

373 Short-term 4-week UE2316 treatment decreased A β plaque number in the cortex and amygdala but
374 not the hippocampus of 15m old Tg2576 mice (Figure 6A). The total plaque number in the brains of
375 Tg2576 mice was 54% lower in UE2316-treated than vehicle-treated mice. Plaque areas were
376 correspondingly reduced (Figure 6B).

377 After chronic 44-week treatment with UE2316 (mice aged 17-18 months) there were a similar number
378 of A β plaques in the cortex and hippocampus as in mice treated with UE2316 for only 4 weeks
379 (Figure 6C). However, there were fewer A β plaques in the brains of the vehicle-treated group from
380 the 44-week study than in the vehicle-treated animals from the 4-week study. In the 44-week study
381 there was no significant effect of UE2316 on number of A β plaques.

382 To explore possible mechanisms mediating the effects of short-term UE2316 treatment on A β plaque
383 burden in the cortex we conducted western blot analyses of selected proteins involved in A β
384 generation and metabolism, the expression of which are known to be regulated by glucocorticoids
385 (Figure 7B). UE2316 treatment did not modulate BACE protein expression in either WT or Tg2576
386 mice (Table 2). Nor did UE2316 alter ADAM10, a metalloproteinase possessing α -secretase activity
387 involved in the non-amyloidogenic pathway of APP processing (29) (Table 2). However, UE2316
388 significantly increased, by 31% and 34% respectively, insulin degrading enzyme (IDE) protein
389 expression in the cortex of both control and Tg2576 mice (Table 2). No difference in IDE expression
390 was found in the brains from mice treated with UE2316 for 44 weeks (IDE to beta-tubulin ratio:
391 vehicle: 0.016 ± 0.004 vs UE2316: 0.011 ± 0.002 ; Student's t-test, $p=0.22$).

392 PSD95 and synaptophysin, markers of synaptic density (30,31), were unaffected by UE2316 (Table
393 2). In addition, microglial density was not increased as evidenced by no change in Iba1 levels (32)
394 (Table 2). CD31 staining was also conducted to probe for potential changes in cerebral vascular

density, as 11 β -HSD1 null mice exhibit enhanced angiogenesis (33), but no effect of UE2316 was observed (Table 2).

Discussion

We have generated UE2316, which is a potent and selective inhibitor of 11 β -HSD1 in mouse brain. UE2316 administered either systemically or directly to the brain induces improvements in memory in cognitively impaired rodents. In aging mice the effects of UE2316 recapitulate those of other selective 11 β -HSD1 inhibitors (15, 16) and provide further evidence that short term inhibition of 11 β -HSD1 in the brain improves memory impairments associated with aging. Moreover, our data demonstrate that these improvements are associated with inhibition of 11 β -HSD1 specifically in the brain since mice treated with icv administration with sub-systemic doses display memory improvements comparable to those observed in mice treated systemically. The effects are evident across a range of behavioral tasks that involve the hippocampus, in contrast with the attenuation of contextual fear-associated memory that we previously reported with UE2316 which is likely mediated in different brain regions (19). It is likely that these short term improvements in memory are due to the effects of reduced intracellular corticosterone in regions of the brain such as the hippocampus, which are sufficient to reverse the memory-impairing effects of glucocorticoids in aged mice (17). Structural changes to the hippocampus, such as synaptic and dendritic atrophy may be reversed by reduced intracellular glucocorticoid levels over a period of hours and weeks respectively, and could be responsible for the short term memory improvements observed in these studies (34,35). However, we also observed significant improvements in memory with long term 11 β -HSD1 inhibition which may be mediated by structural hippocampal changes. It should be noted that behavioral testing was only carried out in male mice and the exploration of any potentially sexually dimorphic effects of 11 β -HSD1 inhibition will require separate, comparative studies in male and female mice.

There is now substantial evidence from studies in rodents and in humans that reductions in 11 β -HSD1 activity in the brain provides beneficial effects on the cognitive decline associated with aging (15, 16,

18, 23). However, to date no studies have been published that assess the effects on 11 β -HSD1 inhibition in rodent models of AD. We found that short term (29 days) treatment of already cognitively-impaired 14-month old Tg2576 mice with UE2316 led to improvements in memory during the passive avoidance task. UE2316 also improved latency in wild type mice but to a lesser extent than in Tg2576 mice. Our data also demonstrate that long term inhibition of 11 β -HSD1 in Tg2576 mice maintains cognitive performance with aging since age-matched mice without UE2316 treatment are cognitively impaired in tests performed from 16 months of age onwards. Moreover, cognitive improvement with 11 β -HSD1 inhibition is maintained in the presence of significant Alzheimer's pathology.

In the Tg2576 mouse strain, A β plaques have been shown to develop from 9 months of age onwards, associated with impairments in cognitive ability in memory tests from 10-12 months (25). As expected, when we examined the brains of the Tg2576 mice A β plaques were observed in the cortex, amygdala and hippocampus. Short term UE2316 treatment substantially decreased A β plaque number and area in the cortex and amygdala of Tg2576 mice. In contrast, in a separate cohort of mice from which those with retinal impairment were excluded, we observed less A β plaque burden and no statistically significant effects of chronic UE2316 administration. This likely reflects the differences in animals with and without visual impairment. Overall, the results suggest that treatment with UE2316 has a disease-modifying effect on amyloid plaque deposition in Tg2576 mouse brains by impairing plaque accumulation. However, our findings dissociate cognitive improvement from A β plaque pathology after chronic treatment, suggesting the mechanism of improved cognition with 11 β -HSD1 inhibition is not mediated solely through reduced plaque burden. Whatever the mechanism, the effect on cognition is likely associated with lowered intracellular glucocorticoid levels in the brain, and the consequent altered balance of glucocorticoid and mineralocorticoid receptor action (23).

Additionally, we observed an increase in cortical IDE protein levels with short term UE2316 treatment. This increase may explain, in some part, the reduction in plaque numbers and plaque area in the drug-treated mice, as previous studies have demonstrated that overexpression of IDE or neprilysin in the neurons of transgenic mice significantly reduced brain amyloid beta levels and

slowed or completely prevented amyloid plaque formation in APP TG mice (36), while IDE null mice have excess cerebral accumulation of A β (37). In humans, genome-wide association studies report a higher susceptibility to AD in Finnish patients with polymorphisms of IDE, suggesting that the rate of A β degradation may be an important factor in the development of human AD (38). However, the lack of IDE induction with chronic UE2316 administration, in the face of persisting benefits for cognitive function, suggests that other pathways are involved in cognitive protection with 11 β -HSD1 inhibition. Alternatively, Tg2576 mice selected for intact vision may be relatively resistant to glucocorticoid effects on IDE and A β turnover.

These pre-clinical results support the concept that 11 β -HSD1 inhibition may be efficacious for memory impairments not only with aging, but also in AD. A recent phase 2 clinical trial in patients with mild-moderate AD was halted, however, when the selective 11 β -HSD1 inhibitor ABT-384 failed to show non-inferiority against donepezil for the primary endpoint of ADAS-Cog score (39).

Although a pharmacodynamic study of ABT-384 using stable isotope d4-cortisol tracer has been reported (40), it remains uncertain whether ABT-384 inhibited 11 β -HSD1 adequately in brain since: data were presented for only two control subjects without administration of ABT-384; after ABT-384 administration, d3-cortisol levels (generated by 11 β -HSD1) (41) were very low in plasma, consistent with systemic enzyme inhibition, and this may account for the undetectable levels of d3-cortisol in CSF; and the maximum CSF concentrations of ABT-384 achieved, which were present for only a short time after dosing, were not high enough to inhibit 11 β -HSD1 by more than 10%, according to published potency of the compound. The benefits of 11 β -HSD1 inhibition may only be apparent in the treatment of early disease, when the combination of symptomatic cognitive improvement and potential for disease modification we have observed in the mouse model of AD may be most useful.

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References

(1) Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NPV, Thakur M, McEwen BS, Hauger RL, Meaney MJ. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci* 1998; 1:69–73.

(2) Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE. Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biol Psychiatry* 1999; 46:1595-1602.

(3) MacLulich AM, Deary IJ, Starr JM, Ferguson KJ, Wardlaw JM, Seckl JR. Plasma cortisol levels, brain volumes and cognition in healthy elderly men. *Psychoneuroendocrinol* 2005; 30:505-515.

(4) de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005; 6:463-475.

(5) Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res* 1990; 531:225-231.

(6) Lupien, SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behavior and cognition. *Nat Rev Neurosci* 2009; 10:434-445.

(7) Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis. *Psychoneuroendocrinology* 2013; 38:1-11.

(8) Peskind ER, Wilkinson CW, Petrie EC, Schellenberg GD, Raskind MA. Increased CSF cortisol in AD is a function of APOE genotype. *Neurology* 2001; 56:1094-1098.

495 (9) Cernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, Morris JC. Plasma cortisol
496 and progression of dementia in subjects with Alzheimer-type dementia. *Am J Psychiatry* 2006;
497 163:2164-2169.

498 (10) Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM. Glucocorticoids increase
499 amyloid- β and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci* 2006; 26:9047-
500 9056.

501 (11) Wang Y, Li M, Tang J, Song M, Xu X, Xiong J, Li J, Bai Y. Glucocorticoids facilitate astrocytic
502 amyloid- β peptide deposition by increasing the expression of APP and BACE1 and decreasing the
503 expression of amyloid- β -degrading proteases. *Endocrinology* 2011; 152:2704-2705.

504 (12) Chapman KE, Holmes MC, Seckl JR. 11 β -hydroxysteroid dehydrogenases: intracellular gate-
505 keepers of tissue glucocorticoid action. *Physiol Rev* 2013; 93:1139-1206.

506 (13) Holmes MC, Carter RN, Noble J, Chitnis S, Dutia A, Paterson JM, Mullins JJ, Seckl JR, Yau JL.
507 11 β -hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus
508 and parietal cortex and causes memory impairments. *J Neurosci* 2010; 30:6916-6920.

509 (14) Yau JL, Noble J, Kenyon CJ, Hibberd C, Kotelevtsev Y, Mullins JJ, Seckl JR. Lack of tissue
510 glucocorticoid reactivation in 11 β -hydroxysteroid dehydrogenase type 1 knockout mice
511 ameliorates age-related learning impairments. *Proc Natl Acad Sci USA* 2001; 98:4716-4721.

512 (15) Sooy K, Webster SP, Noble J, Binnie M, Walker BR, Seckl JR, Yau JLW. Partial deficiency or
513 short term inhibition of 11 β -hydroxysteroid dehydrogenase type 1 improves cognitive function in
514 aging mice. *J Neurosci* 2010; 30:13867-13872.

515 (16) Mohler EG, Browman KE, Roderwald VA, Cronin EA, Markosyn S, Bitner RS, Strakhova MI,
516 Drescher KU, Hornberger W, Rohde JJ, Brune ME, Jacobson PB, Rueter LE. Acute inhibition of 11 β -
517 hydroxysteroid dehydrogenase type-1 improves memory in rodent models of cognition. *J Neurosci*
518 2011; 31:5406-5413.

519 (17) Yau JLW, Wheelan N, Noble J, Walker BR, Webster SP, Kenyon CJ, Ludwig M, Seckl JR.
520 Intrahippocampal glucocorticoids generated by 11 β -HSD1 affect memory in aged mice. *Neurobiol*
521 *Aging* 2014; 36:334-343.

522 (18) Sandeep TC, Yau JL, MacLulich AM, Noble J, Deary IJ, Walker BR, Seckl JR. 11Beta-
523 hydroxysteroid dehydrogenase inhibition improves cognitive function in healthy elderly men and type
524 2 diabetics. *Proc Natl Acad Sci USA*. 2004; 101:6734-6739.

525 (19) Sarabdjitsingh RA, Zhou M, Yau JLW, Webster SP, Walker BR, Seckl JR, Joëls M, Krugers HJ.
526 Inhibiting 11 β -Hydroxysteroid Dehydrogenase Type 1 Prevents Stress Effects on Hippocampal
527 Synaptic Plasticity and Impairs Contextual Fear Conditioning. *Neuropharmacology* 2014; 81:231-236.

528 (20) Cobice F, Mackay CL, Goodwin RJ, McBride A, Langridge-Smith PR, Webster SP, Walker BR,
529 Andrew R. Mass Spectrometry Imaging for Dissecting Steroid Intracrinology within Target Tissues.
530 *Anal Chem* 2013; 85:11576-11584.

531 (21) Webster SP, Seckl JR, Walker BR, Ward P, Pallin TD, Dyke HJ, Perrior TR. (4-Phenyl-
532 piperidin-1-yl)-[5-(1H-pyrazol-4-yl)-thiophen-3-yl]-methanone Compounds and Their Use. *Int PCT*
533 *Application* 2011; WO2011033255.

534 (22) Webster SP, Ward P, Binnie M, Craigie E, McConnell KMM, Sooy K, Vinter A, Seckl JR,
535 Walker BR. Discovery and biological evaluation of adamantyl amide 11 β -HSD1 inhibitors. *Bioorg*
536 *Med Chem Lett* 2007; 17:2838-2843.

537 (23) Yau JL, Noble J, Seckl JR. 11 β -hydroxysteroid dehydrogenase type 1 deficient prevents memory
538 deficits with aging by switching from glucocorticoid receptor to mineralocorticoid receptor-mediated
539 cognitive control. *J Neurosci* 2011; 31:4188-4193.

540 (24) Yau JL, McNair KM, Noble J, Brownstein D, Hibberd C, Morton N, Mullins JJ, Morris RG,
541 Cobb S, Seckl JR. Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-
542 hydroxysteroid dehydrogenase type 1 knock-out mice. *J Neurosci* 2007; 27:10487-10496.

543 (25) Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative
544 memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 1996; 274:99–
545 102.

546 (26) Gil-Bea FJ, Aisa B, Schliebs R, Ramirez MJ. Increase of locomotor activity underlying the
547 behavioral disinhibition in Tg2576 mice. *Behav Neurosci* 2007; 131:340-344.

548 (27) Ishii M, Wang G, Racchumi G, Dyke JP, Iadecola C. Transgenic mice overexpressing amyloid
549 precursor protein exhibit early metabolic deficits and a pathologically low leptin state associated with
550 hypothalamic dysfunction in arcuate neuropeptide Y neurons. *J Neurosci* 2014; 34:9096-9106.

551 (28) Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, Stewart GR,
552 Shafer LL. Intracerebroventricular amyloid- β antibodies reduce cerebral amyloid angiopathy and
553 associated micro-hemorrhages in aged Tg2576 mice. *Proc Natl Acad Sci USA*. 2009; 106:4501-4506.

554 (29) Donmez G, Wang D, Cohen DE, Guarante L. SIRT1 suppresses β -amyloid production by
555 activating the β -secretase gene ADAM10. *Cell* 2010; 142:320-332.

556 (30) Calkins MJ, Manczak M, Mao P, Shirindeb U, Reddy PR. Impaired mitochondrial biogenesis,
557 defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic
558 degeneration in a mouse model of Alzheimer's Disease. *Human Mol Genet* 2011; 20:4515-4529.

559 (31) McClean PL, Parthsarthy V, Faivre E, Hölscher C. The diabetes drug Liraglutide prevents
560 degenerative processes in a mouse model of Alzheimer's disease. *J Neurosci* 2011; 31:6587-6594.

561 (32) Willuweit A, Velden J, Godemann R, Manook A, Jetzek F, Tintrup H, Kauselman G, Zevnik B,
562 Henriksen G, Drzezga A, Pohlner J, Schoor M, Kemp JA, von der Kammer H. Early-onset and robust
563 amyloid pathology in a new homozygous mouse model of Alzheimer's disease. *PLoS One* 2009;
564 4:e7931.

565 (33) Small GR, Hadoke PWF, Sharif I, Dover AR, Armour D, Kenyon CJ, Gray GA, Walker BR.
566 Preventing local regeneration of glucocorticoids by 11 β -hydroxysteroid dehydrogenase type 1
567 enhances angiogenesis. *Proc Natl Acad Sci USA* 2005; 102:12165-12170.

568 (34) Chen Y, Rex CS, Rice CJ, Dubé CM, Gall CM, Lynch G, Baram TZ. Correlated memory defects
569 and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone
570 signalling. *Proc Natl Acad Sci USA* 2010; 107:13123-13128.

571 (35) Conrad CD, LeDoux JE, Magarinos AM, McEwen BS. Repeated restraint stress facilitates fear
572 conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behav Neurosci* 1999;
573 113:902-913.

574 (36) Leissring MA, Farris W, Chang AY, Walsh DM, Wu X, Sun X, Frosch MP, Selkoe DJ.
575 Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary
576 pathology, and premature death. *Neuron* 2003; 40:1087-1093.

577 (37) Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE,
578 Selkoe DJ, Guenette S. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta
579 protein, and the beta amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci USA*
580 2003; 100:4162-4167.

581 (38) Vepsäläinen S, Helisalmi S, Mannermaa A, Pirttilä T, Soininen H, Hiltunen M. Combined risk
582 effects of IDE and NEP gene variants on Alzheimer Disease. *J Neurol Neurosurg Psychiatry* 2009;
583 80:1268-1270.

584 (39) Marek GJ, Katz DA, Meier A, Greco IV N, Zhang W, Liu W, Lenz RA. Efficacy and safety
585 evaluation of HSD-1 inhibitor ABT-384 in Alzheimer's disease. *Alzheimers Dement* 2014; 10: S364-
586 S373.

587 (40) Katz DA, Liu W, Locke C, Jacobson P, Barnes DM, Basu R, An G, Rieser MJ, Daszkowski D,
588 Groves F, Heneghan G, Shah A, Gevorkyan H, Jhee SS, Ereshefsky L, Marek GJ. Peripheral and

central nervous system inhibition of 11 β -hydroxysteroid dehydrogenase type 1 in man by the novel inhibitor ABT-384. Transl Psychiatry 2013; 3:1-7.

(41) Andrew R, Smith K, Jones GC, Walker BR. Distinguishing the activities of 11 β -hydroxysteroid dehydrogenases in vivo using isotopically labelled cortisol. J Clin Endo and Met 2002; 87:277-285.

Tables.

Table 1: Treatment table.

Table 2: UE2316 increased IDE protein levels in the cortex of control and Tg2576 mice. Data are mean \pm SEM from Western blot densitometry, normalized to beta-tubulin. 2-way ANOVA analysis was performed and the treatment effect of UE2316 is shown. For IDE * p <0.04 for effect of UE2316 within each genotype by post hoc Fisher's LSD tests.

Figures and Legends.

Figure 1: UE2316 Characteristics. A: Structural comparison of UE1961 and UE2316 B: Potency and selectivity of UE2316. C: Male C57BL6 mice were treated with a single 10 mg/kg oral dose of UE2316 (n=3 animals per time point) and inhibition by scintillation proximity assay was assessed 1, 4 and 6 hours post dosing and expressed as % inhibition compared with values obtained in vehicle-treated mice. (One-way ANOVA, p =0.009; Bonferroni's post-hoc comparisons, * p <0.05 vs vehicle).

Figure 2: UE2316 improved spatial and fear-associated memory in aged C57Bl6 mice. Aged 22 month-old C57Bl6 mice were treated with 0 (n=6), 5 (n=8) or 15 (n=8) mg/kg/day UE2316 for 23 days via SC implanted osmotic minipumps. A: Spatial memory was assessed by Y maze on day 10 of treatment. The initial 1 minute ITI was performed prior to surgery. Treatment with 15 mg/kg/day UE2316 increased the time spent in the novel arm in the 2 hour ITI compared to vehicle treated animals (* p <0.05 by Student's t-test with Bonferroni correction), and a trend for improvement was

seen with the 5 mg/kg/day dose. B: Passive avoidance was analyzed on days 13 and 14 of treatment. Both 5 mg/kg/day (*p=0.02 vs vehicle by Student's t-test) and 15 mg/kg/day (*p=0.03 by Student's t-test) UE2316 improved latency in the retention trial compared to the vehicle treated group (by two-way repeated measures ANOVA drug interaction with training vs retention p<0.05). C: Similarly aged C57Bl/6 mice were treated with an infusion of either vehicle (artificial CSF) (n=9) or 100ng/h UE2316 (n=8) via ICV cannulas. Spatial memory was assessed by Y maze on day 9 of treatment. The initial 1 minute ITI was performed prior to surgery. Treatment with UE2316 increased the time spent exploring the novel arm during the 2 hour ITI compared with vehicle-treated controls (**p=0.005 by Student's t-test).

Figure 3: UE2316 improved fear-associated behaviour in the passive avoidance test in Tg2576

mice. Tg2576 and wild type mice (n=10 per group) were treated with vehicle or UE2316 at 10 mg/kg/day by SC Alzet minipump infusion from the age of 14 months for 29 days. A: Tg2576 mice were assessed in the passive avoidance task on days 27 and 28 of drug treatment. The latency to enter the dark compartment was assessed in the training trial (pre) and the retention trial (post). UE2316 increased latency to enter the dark compartment 6 hours post shock (2-way repeated measures ANOVA drug effect p<0.04; **p<0.01 by Student's t test) to a greater extent in Tg2576 mice (by an increment of 171.6 ± 28.0 seconds compared to 42.6 ± 21.5 seconds in vehicle-treated mice, **p=0.004 by Student's t-test). B: Open-field was performed after 23 days of treatment in vehicle and UE2316 treated wild type and Tg2576 mice. The percentage of time during the 5 minute trial that was spent in the inner zone of the open field apparatus was measured. There was no significant effect of treatment or genotype. C: Spontaneous alternation was assessed at day 26 of treatment. There was a trend for increased alternation with UE2316 treatment (2-way ANOVA; treatment: p=0.06).

Figure 4: Long term administration of UE2316 improves cognition in Tg2576 mice.

Tg2576 mice were treated with either control diet (RM1, n=16) or RM1 supplemented with 175 ppm UE2316 for an estimated dose of 30 mg/kg/day (n=32) from the age of 6- 7 months for 57 weeks. A: Passive avoidance was analysed after 15 weeks of treatment with vehicle (n=13) or UE2316 (n=23). Both groups exhibited significantly increased latency to enter the dark compartment in the retention test,

indicating preserved cognitive function but there was no effect of UE2316 (by two-way ANOVA training vs retention effect $p<0.001$, drug effect $p=0.26$; by Student's t-test $*p=0.03$, $***p=0.0007$). B: Passive avoidance was retested after 41 weeks of treatment with vehicle ($n=13$) or UE2316 ($n=23$) in chow. UE2316 but not vehicle treated mice demonstrated a significant increase in latency to enter the dark compartment in the retention test (by Student's t-test $**p=0.004$).

Figure 5. Behavior of Tg2576 mice following long term administration of UE2316. A: Open field was performed in control (vehicle: $n=5$, UE2316: $n=5$) and Tg2576 mice (vehicle: $n=13$, UE2316: $n=23$) after 38 weeks of treatment with either normal chow or diet containing UE2316. The Tg2576 mice spent more time in the inner zone than their wild type counterparts; however, there was no effect of drug administration (by two-way ANOVA, $*p<0.05$). B: Spontaneous alternation was assessed in Tg2576 mice after 39 weeks of vehicle ($n=13$) or UE2316 treatment ($n=23$). UE2316-treated mice exhibited a significant increase in percent alternation compared to control mice (by Student's t-test $*p=0.04$). C: The spatial Morris Water Maze test was performed after 52 weeks of treatment. Mice that were able to find the platform in a visible platform test were tested in their ability to find the submerged platform using spatial cues located around the testing room. UE2316 treated mice ($n=9$) exhibited significant decreases in latency to find the hidden platform across the testing period compared to vehicle treated animals ($n=6$) (2-way repeated measures ANOVA, drug effect $p=0.02$ and interaction of drug with time $p<0.01$; by Student's t-tests $*p<0.05$ at days 3, 5 and 6).

Figure 6: Effect of short- and long-term UE2316 administration on amyloid plaque burden in Tg2576 mice. A: Short term plaque number. 6E10 positive amyloid plaques were counted in at least 5 non-sequential sections per mouse treated for 29 days with vehicle or UE2316 ($n=10$ per treatment) via SC minipumps using the KS300 imaging program and the total number of positive plaques was expressed per area of the brain. UE2316 had no effect on plaque number in the hippocampus but decreased 6E10 staining in the cortices (Student's t-test, $**p=0.002$), amygdala (Student's t-test, $*p=0.05$) and whole brain (Student's t-test, $*p=0.01$) in comparison to vehicle. B: Short term plaque area. Total plaque area of short term (29 day) treated mice was measured using the KS300 imaging program and was expressed as plaque area divided by the total area of the brain region in question.

The total plaque area in the cortex was decreased by UE2316 in comparison to vehicle (Student's t-test, *** $p=0.0001$). C: Long term plaque number. 6E10 positive amyloid plaques were counted in at least 5 non-sequential sections per mouse treated for 44 weeks with vehicle or UE2316 ($n=5$ per treatment) in the diet using the KS300 imaging program and the total number of positive plaques was expressed per area of the brain. UE2316 had no statistically significant effect on plaque number in the hippocampus or cortex in comparison to vehicle.

Figure 7: Effects on brain pathology in Tg2576 mice treated with UE2316. A: Representative brain sections from Tg2576 mouse showing amyloid plaques in brain regions stained with 6E10 antibody. (a) cortex, vehicle treated (b) cortex, UE2316-treated (c) amygdala, vehicle-treated (d) amygdala, UE2316-treated (e) hippocampus, vehicle-treated (f) hippocampus, UE2316-treated. B: Representative western blot of cortex protein (30 μ g/sample) from 29 day treated mice. Quantitation was performed using the Odyssey Infrared imaging system and adjusted for beta-tubulin. IDE levels were increased in UE2316 treated cortices compared to vehicle treated tissues in both wild type and Tg2576 animals (see Table 1).

Supplementary Data.

Supplementary Figure 1: Effects of short-term (29 day) treatment with UE2316 in wild type and Tg2576 mice on food intake and body weight. Wild-type and Tg2576 mice were treated via 2 subcutaneously implanted Alzet minipumps with either vehicle or 10 mg/kg/day UE2316 for 29 days ($n=10$ per treatment). A: Body weights were measured weekly in all mice. There was no effect of UE2316 on body weight although the Tg2576 mice weighed less than the controls (two-way repeated measures ANOVA, genotype effect $p<0.001$ and interaction with time $p=0.001$). B: Food was weighed weekly in all mice. Food intake was averaged as grams of food consumed/day. Tg2576 mice consumed more food but there was no effect of UE2316 (two-way repeated measures ANOVA genotype effect $p=0.004$).

Supplementary Figure 2: Effects on food intake and body weight with long-term inhibition of 11 β -HSD1 in Tg2576 mice by UE2316 supplemented diet. A: Estimated daily drug dosage was calculated weekly by measuring the food intake and comparing with body weight. The dose of UE2316 remained constant throughout the experiment. B: Individually housed mice treated with either control diet (RM1; n=16) or RM1 diet supplemented with 175 ppm UE2316 (RM1 + UE2316; n=32) were weighed weekly. 5 animals per group were culled after 45 weeks and the remainder continued until 57 weeks. By repeated measures ANOVA, there was an interaction of drug treatment with time only before 45 weeks ($p<0.01$) with UE2316 treated animals tending to be lighter in the early weeks of the study. C: Food intake was measured weekly in both groups and was averaged as grams of food/day. Food intake was higher in mice fed diet supplemented with UE2316 in the first few weeks of the study (by repeated measures ANOVA, drug interaction with time $p<0.001$; by Student's t-test $*p<0.05$ at week 10).

Supplementary Figure 3: Effects on behavior and brain pathology in Tg2576 mice treated with UE2316. The water maze probe test was performed 24 hours after the final spatial water maze trial. UE2316-treated Tg2576 mice (n=9) spent significantly more time exploring the target quadrant of the water maze than the vehicle treated mice (n=6) ($*p=0.02$).